36 Vardenafil corrects chloride transport across F508del-CFTR intestinal mucosa

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Objectives: Nasal potential difference measurements have previously shown that vardenafil, a phosphodiesterase type 5 inhibitor, improves CFTR-mediated chloride secretion across the nasal mucosa of mice homozygous for the F508del mutation (CF). This work aimed at studying the potential of vardenafil to rescue CFTR function across the rectal mucosa, representative of the GI tract.
Methods: Distinct rectal potential difference (RPD) profiles were obtained in CF and normal homozygous wild-type mice (WT). Sodium absorption, measured by the response of 10^{-4}M amiloride (in the presence of 5×10^{-3} M barium to block potassium channels), was much higher in CF (40.2±4.0 mV) than in WT mice (20.0±1.8 mV; $p < 0.001$). Chloride secretion recorded in the presence of chloride-free solution and 10^{-5} M forskolin was twice as low in CF (–4.2±0.5 mV) as in WT mice (–9.4±0.9 mV, $p = 0.002$). Heterozygous mice showed preserved sodium transport (22±1.9 mV) but reduced chloride secretion (–5.4±1.5 mV). Chloride secretion was restored in CF mice treated with a single ip dose of 0.14 mg/kg vardenafil; values reached after treatment (–9.3±1.2 mV) were similar to those obtained in untreated WT mice.
Conclusion: Our findings pointed out the rectal mucosa as an additional target tissue to study *in vivo* ion transport abnormalities. The RPD test discriminates between CF and non-CF and can be used to investigate the efficacy of therapeutic strategies to rescue CFTR function. As for the airways, vardenafil restores chloride secretion across the GI epithelium. Immunolocalization of CFTR protein in colon tissue preparations are under investigation in KO, CF and WT mice treated or not-treated with vardenafil.

37 Carbachol and forskolin stimulated bicarbonate transport across human rectal biopsies is dependent on functional CFTR

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The measurement of electrolyte transport through rectal biopsies is a useful *ex vivo* tool to diagnose Cystic Fibrosis (CF). However, the role of bicarbonate transport by colonic epithelia is only poorly understood. For the present study rectal biopsies were obtained from pediatric CF and non-CF patients and subjected to measurement of transepithelial voltage (V_{te}) and resistance using a modified perfused Ussing chamber. Stimulation of anion secretion by carbachol (CCH) led to a transient lumen negative deflection of V_{te} in non CF tissues and a positive deflection in tissues obtained from CF patients. Perfusion of the tissue with indomethacin in the absence of bicarbonate led to a time dependent reversal of the CCH response similar to that observed in biopsies obtained from CF patients that were not treated with indomethacin. The effect of indomethacin could be abolished when the tissue was stimulated with forskolin/IBMX (FSK/IBMX) indicative of an inhibition of endogenous cAMP production by indomethacin. Much to our surprise the inhibitory effect of indomethacin on the response to CCH could be abolished when the tissue was perfused with a solution containing bicarbonate. Addition of bicarbonate in the presence of FSK/IBMX further enhanced secretion and was unaffected by the serosal addition of bumetanide. In tissue obtained from CF patients neither the effect by CCH nor that by FSK/IBMX could be modulated by the addition of bicarbonate. We conclude that chloride and bicarbonate ions utilize different pathways in CCH and FSK/IBMX stimulated colonic biopsies and that the transport of both anions is dependent on functional CFTR.
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38 Combination of *CFTR* mutations producing frequent complex alleles with different clinical and functional outcomes

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Genotype–phenotype correlations in CF may be complicated by phenotype variability and existence of complex alleles. We aimed to elucidate the clinical significance of complex alleles associating p.Gly149Arg, p.Asp443Tyr, p.Gly576Ala and p.Arg668Cys by a collaborative genotype–phenotype correlation study in 153 patients, and structure-function analyses for the single and natural complex mutants, p.[Gly576Ala;Arg668Cys], p.[Gly149Arg;Gly576Ala;Arg668Cys] and p.[Asp443Tyr;Gly576Ala;Arg668Cys]. Only 3 subjects had classical CF and all carried p.Gly149Arg in the triple mutant, whereas genotypes combining other mutants that do not contain p.Gly149Arg were observed in patients with moderate phenotypes, mostly CBAVD. Functional studies showed that: p.Gly149Arg is a severe misprocessing defect; p.Asp443Tyr moderately alters CFTR maturation; p.Gly576Ala and p.Arg668Cys mildly alter CFTR chloride conductance. The overall results consistently show the contribution of p.Gly149Arg to the CF phenotype whereas p.Arg668Cys, p.[Gly576Ala;Arg668Cys] and p.[Asp443Tyr;Gly576Ala;Arg668Cys] should be considered as alleles associated with CFTR-related disorders.

39 Pathological role of the calpain/calpastatin system in cystic fibrosis

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Objectives: To establish the involvement of the calpain/calpastatin system, the components of the calcium-dependent proteolysis, in cystic fibrosis (CF), we have analyzed peripheral blood mononuclear cells (PBMC) of 12 CF patients (F508del-CFTR homozigotes) in which the alteration in levels and localization of F508del-CFTR is identical to that reported in airway epithelial cells models.
Methods: The role of calpain in CF has been studied by immunoblot analysis, immunoprecipitation, confocal microscopy, and assay of intracellular protease activity.
Results: Whereas in PBMC from controls, the basal calpain activity is almost undetectable, in CF-PBMC the protease activity is significantly measurable, due to an increase in $[Ca2+]_i$ and a decrease in the level of both calpastatin protein and inhibitory efficiency. The imbalance in regulation of the calpain/calpastatin system favors the activation of the protease, explaining the presence of the digested CFTR form. As a result of such protease activation, NHERF-1, a partner of CFTR in its functional complexes, is also present in PBMC of CF-patients in a digested form, showing a mass of 20 kD. This covalently modified NHERF-1 is completely absent in controls as well as in heterozygous CF parents. Ezrin, another component of the CFTR functional clusters, is also degraded in CF PBMC.
Conclusion: Our observations are indicating that calpain digests different components of the CFTR-generated protein complexes, promoting their removal from plasma membranes and accumulation into cytoplasm. Restoration of the intracellular calpain regulation with new synthetic inhibitors could provide a new therapeutic approach to CF.